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Quality Evaluation of Prepared Zobo Concentrate Sweetened with Inverted Sugar Syrup and Sodium Cyclamate

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Received: June 14, 2022; **Published:** June 29, 2022

Abstract

In this study, triple strength Zobo concentrate (ZC) was prepared using purple calyx of Sorrel by Hurdle Technology; and aseptically packaged in ambered-coloured containers for spray drying. The prepared samples of ZC were sweetened with inverted sugar syrup (coded 617) and sodium cyclamate (coded 615) were subjected to sensory, physico-chemical and microbial analyses. The physico-chemical properties of both samples for the pH ranges from 4.1 for sample 615 (i.e. ZC sweetened with Sodium cyclamate) and 4.3 for sample 617 (i.e. ZC sweetened with inverted sugar syrup); and the Brix content of the sample coded 615 was 5.8% and 3.8% for the sample coded 617, respectively. And the results of the microbial analysis indicated that both samples are safe for human consumption because both samples microbiological result is < 1.0×10^1 cfu/ml, which is within microbial limits (10^3 cfu/g) for ready-to-eat (RTE) foods. The results for the Sensory evaluation conducted for T-test for the two samples 615 and 617 are significantly not different ($P < 0.05$) from each other in terms of colour, taste (mouth-feel) and overall acceptability; while samples 615 and 617 show significant difference in terms of aroma and sweetness ($P < 0.05$). The results of the Ranking test suggested that both samples were found to be significantly different with respect to sweetness ($P < 0.05$). This study suggests that the preparation of triple strength ZC was achieved using Hurdle technology for the purpose of spray drying process.

Keywords: *Dark Purple Calyx; Hurdle Technology; Inverted Sugar Syrup; Zobo Concentrate*

Introduction to the Study

The quest to produce shelf stable Zobo concentrate (ZC) of triple strength sweetened with Inverted sugar syrup (i.e. nutritive sweetener) and Sodium Cyclamate (i.e. calorie-free sweetener) prompted this study, this is in an attempt to prepare instant Zobo drink (i.e. in powdery form) by spray drying process; hence new technology (i.e. Hurdle) for ZC preparation from Purple Sorrel calyx needs to be redirected towards new research [8,11,32,35-38,59]. ZD also known as Sorrel drink is a non-alcoholic beverage produced from the dried calyces of Red and/or Purple variety of matured *Hibiscus sabdariffa* by both the boiling, or steeping methods and then filtration of the liquor before being sweetened with either nutritive or non-nutritive sweetening agents [46,50]. In Nigeria, liquid Zobo drink is produced from Sorrel calyces which is indigenous to the tropics; and the English, French and Arab speaking countries refers to it as *Sorrel, Orselle and Karkade,* respectively [46]. Furthermore, Nutritionists have found Sorrel calyces to contain excellent amount of Calcium, Niacin, Riboflavin and Iron [48]. Another study reported the feasible and promising medicinal potentials of Sorrel calyces by managing patients suffering from Type-2 diabetes because of its hypoglycemic and hypo-lipidemic effect; hence calyces of Sorrel can improve the lipid profiles of the

diabetic's patient [22]. A study conducted by [48] has highlighted that three cultivated varieties of calyces are recognized in Nigeria which are the Red, Purple and Cream, respectively; and it has been established that the purple variety of calyx are of two sub-varieties including extremely dark purple and light dark purple; however the Red and Purple varieties of calyces are used for the production of Zobo drink. And Zobo drink is usually prepared at home by housewives or those families on low income to support immediate and extended family members; and it is mostly sold at home, school premises, markets, restaurants and motor-parks especially during the summer when the temperature goes above 37°C [6].

Having known that zobo drink is being prepared from different varieties of Sorrel calyx, specifically red and purple varieties; this study seeks to prepare Zobo Concentrate (ZC) from the extremely dark purple Sorrel calyx by using Hurdle technology of concentration, pasteurization, acid regulation and sweetening approach.

Introduction to sorrel calyces used for preparation of zobo drink

True Roselle from the family *Malvaceae* are of two main types namely *Hibiscus sabdariffa Variety altissima* and *Hibiscus Sabdariffa Variety sabdariffa* [42,48]. The more important economically is *Hibiscus sabdariffa variety altissima*; it is an erect, sparsely-branched annual to 16ft (4.8m) high, which is cultivated for its jute-like fiber in India [41]. The stems variety is green or red and the leaves are green non-flesh, spiny and not used for food [41,48]. The other distinct type of 'Roselle' *Hibiscus Sabdariffa Variety sabdariffa* embraces shorter and bushy form *Hibiscus Sabdariffa Variety sabdariffa* is used in the production of sorrel calyces use for the production of ZD and the major two species used for ZD production are the red and purple calyces [46,47]. [46] also highlighted that three botanical varieties are recognized in the northern part of Nigeria including the Red, Purple and Cream calyces, and of recent the purple are of two variety including extremely dark and lightly dark purple; however, the Red and Purple varieties are used for the preparation of Zobo drink in Nigeria [46,56]. The nutritional composition of fresh calyces of the plant differs from different studies, probably due to different varieties/genotype, plant environments, and harvesting conditions. [46] reported that 100g of the calyces gave protein (1.9g), fat (0.1g), carbohydrates (12.3g), and fibre (2.3g). The calyces are a good source of ascorbic acid (14 mg), iron (57 mg) and β-carotene (300 µg) and this plant is rich in antioxidants [15]. Wong PK., *et al*. [62] found Roselle to contain high contents of vitamins, protein, carbohydrate, and vitamin C. Furthermore, [7] listed the following as nutritional constituents of 100g fresh calyces: fats 2.61g, fibre 12.0g, ash 6.9g, calciun12.63 mg, protein 1.145g, phosphorus 2732 mg, iron 8.98 mg, carotene 0.029 mg, niacin 3.765 mg, thiamine 0.117 mg, ascorbic acid 1.7 mg and riboflavin 0.277 mg [56,58,61,62].

Many reports confirmed that the calyx is loaded with a high content of calcium, niacin, riboflavin, iron and vitamin C is nine (9) times stronger than an Orange. The United State Department of Agriculture reported that 100g of calyces contain 11.31g of carbohydrate, 0.64g of fat, and 0.96g of protein. Similarly, Roselle has been reported to contain biological active chemical substances that have curative properties. [47,60] reported that the major bioactive constituents of the calyces that have a pharmacological function are flavonoids, organic acids, anthocyanin, and polysaccharides. The phytochemicals in Roselle calyces according to contained flavonoids 20.08%, tannins 17.00%, saponins 0.96%, phenols 1.10%, alkaloids 2.14% and glycosides 0.132%, which must have given it its pharmacological properties. Extract of calyces has been reported to reduce the viscosity of the blood; it also has antimicrobial, diuretic, febrifugal, antihypertensive, and anti-helminthic properties. Similarly, earlier studies demonstrated that Roselle calyces contain high contents of anthocyanin and phenolic compounds. Anthocyanins are a group of plant pigments that are responsible for the attractive colours of many fruits and flowers including calyces of red Roselle. Six anthocyanins are common in fruits and vegetables; these are Pelargonidin, Cyanidin, Delphinidin, Malvidin, Peonidin and Petunidin. Anthocyanins are important quality indicators in plants. Research reports have shown that anthocyanin has many medicinal benefits, among which are antioxidant, anti-carcinogenic, antimicrobial, anti-inflammatory, cardio-protective, and hepato-protective properties. To provide support to our body defense mechanism, our diet must contain a sufficient amount of nutrients and antioxidants. Incorporation of antioxidant-rich foods in our daily diet boosts our immune system and it is safer and cheaper than

commercially available antioxidants. Accumulation of free radicals in the body leads to oxidative stress, which is linked to several diseases in the body and can even cause death. Antioxidants are compounds, which inhibit the rate of oxidation of molecules in the body through the obstruction of the multiplication of the chain reaction of free radicals. Natural antioxidants are better than the synthetic ones as they usually have nutraceutical properties. Roselle is one of the plants that have been reported to contain various types of anti-oxidants in huge amount and, hence, possess high pharmacological properties. The red calyces contain anti-oxidants including Hibiscetine, Gossypetin, Sabdaretine, cyanidin 3-sambubioside and delphinidin [47].

Description of Zobo drink

Mohammed FS and Balen DMA [46] described Zobo drink (ZD) is a sparkling red-purple color juice prepared from red or purple calyces of sorrel and it tastes like Cranberry; and it is prepared by extracting the dried or fresh sorrel calyces by either boiling or steeping methods; it is consumed by many people for its ability to quench thirst, stimulating effect and nutritional value. Zobo is a major nutritional drink consumed by several socio-economic classes of people in Nigeria and some other West African countries [52]. But, due to the ease of production and availability of raw material especially the calyx of *Hibiscus sabdariffa*, ZD is a source of livelihood to several families in both Northern and Southern Nigeria especially in the rural areas. Poor sanitary condition at the point of production and contaminated water could put consumers of these products into risk. The quality of ZD depends mainly on the physicochemical constituents of the raw materials, water used in its preparation and the hygienic condition of the processors [47,52,53]. Water is a major resource used in the production of ZD from their raw materials [47,52,53]. Poor quality with regard to both physicochemical (colour, pH, turbidity, total suspended solids, total hardness, total alkalinity salinity, electrical conductivity), heavy metals (lead, cadmium, chromium, iron, zinc, copper, nickel, arsenic) and microbial (total heterotrophic bacteria, total fungi, total coliform and fecal coliforms) could also impact on the overall quality of the ZD [3,5,47,52]. The environment in which ZD is prepared could also influence its safety especially in the perspectives of microbial status. ZD is classified as a nutritious drink consumed by people in Nigeria [47,52]. However, the consumption of local beverages, such as the ZD, could be a potential source of transfer of zoonotic and foodborne pathogens including *Staphylococcosis, Salmonellosis, Brucellosis, Tuberculosis, Shigellosis, Listeriosis*, and *E. coli*, infections [19,47,52 54].

Roselle is used in many folk medicines; although in Nigeria, the dry red calyces are processed into a refreshing non-alcoholic beverage known as Zobo and despite the fact that the popularity of Zobo drink is increasing, one of its greatest limitation for large scale production is that it has a short shelf life of 24 hours if not refrigerated [47,54]. The calyces used for the preparation of the drink have been reported to be a major source of contamination as they housed many microbes which spoil foods particularly fungi and bacteria [44,47,52,54]. Other sources of contamination include the unhygienic states of other materials for preparation (such as water, sweeteners and preservatives), the equipment used, packaging materials, place of preparation, the processors' unhygienic conditions, and poor storage [47,52,54]. These sources are, however, reported to pose lower contamination when compared with the contamination from calyces [47,52,54]. Therefore, there is urgent need to explore various preservation methods that could be employed to extend the shelf life of this product. According to [17,59,60] some chemical preservatives were employed to improve the shelf life of Zobo drink [5,8,19,60]. It was reported that only samples treated with benzoic acid remained organoleptically attractive after 14 days of storage. However, the use of good manufacturing practices (GMP) to prevent the problem with the use of chemical preservatives in food which tend to have adverse effects on the health of consumers has to be put in place [4].

Economic importance and other uses of roselle in different parts of the world

Almost all parts of the Sorrel plants are used for food preparation. The seeds, calyces and the leaves are, however, the most useful (Figure 1).

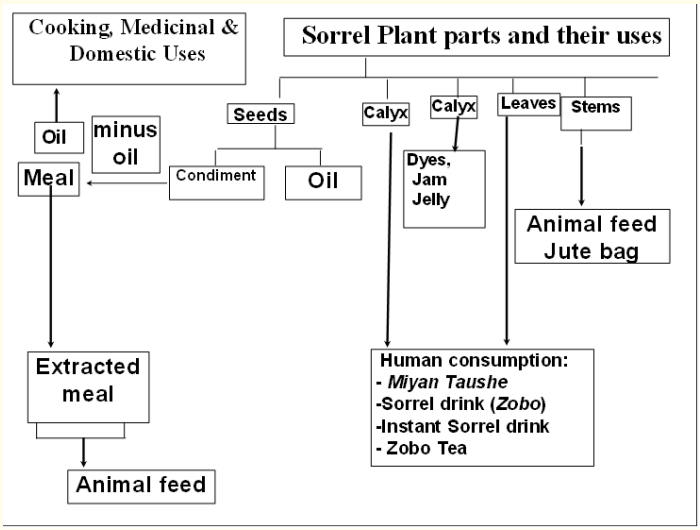

Figure 1: *Uses of sorrel plant products.*

Source: [45].

The seeds, calyces and the leaves of Sorrel plant are used in the preparation of vegetable oil, condiment, varieties of non-alcoholic beverage drink, Jam, Jelly, natural Colourant, Tea, traditional soups and sauces, respectively [45].

Health benefits and safety of roselle drink

The ZD has been reported to have anti-oxidant, anti-hypertensive, anti-hypolipidemic, anti-cancer, anti-bacterial, hepato-protective and anti-stress, anti-diuretic, anti-spasmodic and anti-diarrheal activities. Another study found that Zobo drink affects metabolism, thereby preventing obesity and fat build-up in the liver. Roselle has generally been considered safe as a foodstuff; dosage of 1.5g is recommended for daily consumption [50,51,62]. The safety profile of Roselle is excellent, with no proven adverse reactions. Noteworthy, a study affirmed that, for reducing cholesterol level, studies recommend 1,000 mg dried herb three (3) times daily; 1 cup of the drink two (2) times daily, or 100 mg of standardized extract twice daily and also for hypertension 1 cup of the drink twice daily or dried powdered Roselle extract providing 250 mg anthocyanin per day has been recommended [50].

Materials and Methods

Materials

The materials required for the preparation of Zobo concentrate (ZC) was extremely dark purple sorrel calyx, Inverted sugar syrup and artificial sweetener (sodium cyclamate). Sorrel calyx, granulated sugar and the artificial sweetener were purchased at Katsina central market, Katsina town, Katsina state. Other materials used for the preparation of ZC include: Benzoic acid, Citric acid and potable water. Additional materials used for the production of ZC where weighing balance, a stainless pot, stirrer, thermometer, sieve (1.5 µm sieve size), funnel, measuring cylinder, plastic bowl, spoon, cup, gas cooker, and amber colour PETs containers [38].

Experimental design

Extremely dark purple Sorrel calyx, inverted sugar syrup (ISS) and Sodium cyclamate (SC) were used in the preparation of two samples of ZC, respectively; the ZC was sweetened with ISS and SC; the preparation of samples of ZC was done under hygienic conditions using Hurdle Technology by adopting and modifying the method of [43,44,46]. And Food Grade Benzoic and Citric acids were used as preservative and acid regulator [33]. And Microbiological, physico-chemical and sensory evaluation were conducted on both products [21]. And t-Test (two-tail) and Ranking were used for statistical analysis of all the data ($P < 0.05$) to compare the relationship between the two variables [25-27,31]. All data were recorded in duplicates and results were presented in tables, respectively.

Raw materials, ingredients/recipes formulations

To have a control over the preparation of samples of Zobo concentrate (ZC), unsweetened and sweetened, raw materials and ingredients formulations were established after three trial production methods as presented in table 1-3, respectively.

Raw materials formulation and preparation of zobo concentrate liquor (ZCL)

The preparation of ZCL was done in three stages namely extraction of Zobo liquor by concentration method, production of Inverted Sugar syrup by hydrolysis method and mixing of all ingredients and other additives for the preparation of ZC sweetened with ISS and SC, respectively. The weight of the purple Zobo calyx to potable water for the preparation of Zobo concentrate liquor (ZCL) used as presented in table 1 below.

S/No	Ingredients	Quantity
1	Potable water	3,000 ml
2	Dry Zobo calyx	690g
3	Zobo Liquor	1, 750 ml (58.30% yield)
4	Moisture loss during concentration	1,250 ml (41.70% loss)

Table 1*: Raw materials weights and potable water for preparation of ZC liquor.*

Table 1 presents the weights of raw materials and potable water used for the preparation of Zobo liquor; also it presents the material (Zobo liquor) balance after the completion of the extraction and concentration unit operations. And then formulation for both samples of sweetened ZC was then initiated after multiple trials. Hence, and all the ingredients were accurately measure with the aid of the electronic compact weighing balance (Model KD-BV, China).

And the amounts in percentage was calculated using the formula below:

$$\% = \frac{\text{Weight of individual ingredient}}{\text{Total weights of all ingredients}} \times 100.$$

S/N	Ingredients	Quantity (ml/g)	Percentage
1	Zobo liquor	800 ml	49.847
2	ISS	800 ml	49.847
3	E210	0.525g	0.033
4	E330	4.375g	0.273

Table 2: *Ingredients formulation for zobo concentrate sweetened with ISS.*

Table 2 presents the ingredients formulation for the production of Zobo Concentrate sweetened with inverted sugar syrup (nutritive sweetener).

S/N	Ingredients	Quantity (ml/g)	Percentage
1	Zobo liquor	800 ml	98.32
2	Sodium Cyclamate	8.75g	1.075
3	E210	0.525g	0.065
4	E330	4.375g	0.54

Table 3: *Ingredients formulation for zobo concentrate sweetened with sodium cyclamate.*

Table 3 present the ingredients/recipes formulations for the preparation of two samples of Zobo concentrate sweetened with Sodium cyclamate (non-nutritive sweetener).

Preparation of inverted sugar syrup (ISS)

1,000g of granulated sugar was weighed using a digital weighing balance (Electronic Balance 200, China) and transferred into 3 liters of boiling potable water; the boiling mixture was mixed constantly to avoid lumping and to ensure total dissolving; the dissolved mixture was heated for 30 minutes to ensure partial inversion; and then two drops of concentrated HCl was added into the boiling sugar and stirred continuously to ensure complete inversion (occurrence of hydrolysis); the mixture was boiled for another 35 min and a golden yellowish colour product was obtained and inverted sugar syrup was successfully prepared (Figure 2).

Preparation of zobo concentrate liquor (Unsweetened and sweetened)

Required grams (Table 1) of sorted dark purple sorrel calyx and potable water, were weighed and measured with the aid of a calibrated digital weigh balance (Electronic Balance 200, China) and measuring cylinder. Zobo Concentrate (ZC) was produced using the modified boiling method as described by [46]. Sorrel calyx was sorted, cleaned, weighed and then boiled in potable water at 100°C for 30 minutes; the extracted liquor from Zobo calyx was then sieved using 1.5 µm sieve; and then concentrated at 105°C for 45 minutes (for first concentration) and at 90°C for 60 minutes (for second concentration) and sweetened with already prepared inverted sugar syrup (ISS) and purchased artificial sweetener (sodium cyclamate) (Refer to table 2 and 3 for recipe formulations). And then both sweetened ZC were preserved using Benzoic and Citric acids before being pasteurized at 85°C for 60 seconds, then rapidly cooled using iced water of 0°C for 5 minutes and then packaged in sterilised amber-coloured and readily labelled PETs containers. And finally the prepared samples of ZC were stored at 37°C for further analyses including microbial, sensory and physico-chemical, respectively [46].

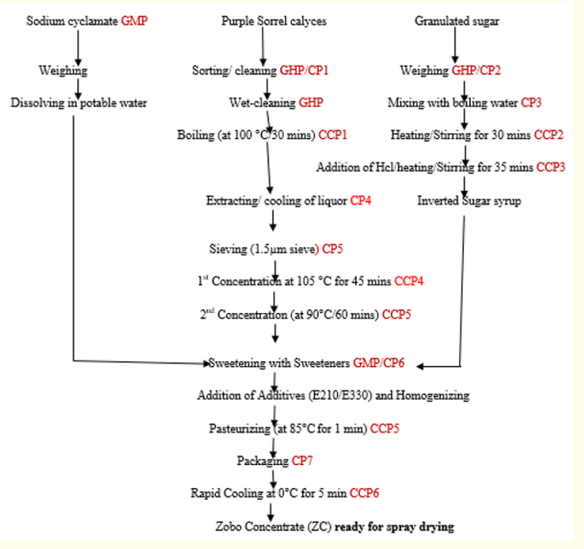

Figure 2: *Process flow diagram for the preparation of zobo concentrate (ZC) by hurdle technology.*
Sources: Adopted and Modified Methods of [10,14,46,50,52].

Packaging of prepared zobo concentrate (ZC) in well labelled PET containers

Packaging of both samples of ZC in a labelled amber-colour PET containers is to protect them from external contact and environment and safely store the Sorrel products at a room temperature. This is to provide excellent protection for the ZC against ultraviolet (UV) light by blocking it [38]. This is because it has been proven scientifically that UV rays can sometimes change the components of the contents of containers as a result of photo-oxidation; also UV rays can encourage spoilage of beverages packaged if the colour is not amber [40]. In addition, amber-colour containers prevents it contents from Blue light with a primary concern of photo-chemical effect on food and bacteria interacting with the food causing spoilage; and as well amber colour can protect potential leaching of the plastic containers into the food product during storage]40]. Amber colour blocks both the UV, Blue and all other lights of wavelength under 450 nano metre making it an ideal for storing light-sensitive food and beverage products [39]. Nonetheless, the matrix effects of UV light, at various levels of wave length and time of exposure, on beverages and foods have been established by a research finding including colour, flavour and nutritional impacts [15]. Hence, the need to use amber colour PET container to package ZC due to its colour and availability of UV light sensitive nutrients including Beta carotene and Iron contents, respectively.

pH determination

10 ml of ZC sample was accurately measured and transferred into a beaker the pH was determined with a previously standardized pH meter (Model 3505, Jenway Ltd, UK). The pH metre was then placed in the beaker containing the solution and the reading was taken and recorded. Each sample reading was taking three times and the average was calculated to ensure an accurate result [2,46,49].

Determination of brix

In determination of Brix of the production a Digital hand-held Pocket Refractometer (Model PAL-1Q 3810-E04, USA) was used by placing a drop of the samples of the RZC on the surface of the Refractometer and its % was read from the side. Each sample reading was taken in triplicate and the average was calculated to ensure an accurate result [2].

Reconstitution ration analysis of the prepared zobo concentrate

In the analysis of the reconstitution ratio of the prepared Zobo concentrate (ZC), equal volume of both samples of the ZC and the potable water were accurately measured and mixed together to obtain a reconstituted Zobo concentrate (RZC) as described but modified by the method of [45]. And the RZC was used for the sensory analyses exercises.

Sensory evaluation of samples of reconstituted zobo concentrate (RZC)

For a successful sensory evaluation exercise, a consent form seeking for sensory judges-participation in sensory evaluation Exercise was designed and distributed to sensory judges that are familiar with Zobo drink. This is because recently sensory panelists are usually informed if they are interested to take part in sensory analysis exercise; this is to initiate and notify the sensory judges prior to the day scheduled for the sensory analysis. Participant information sheet is usually sent to sensory judges for their information, consent seeking and acceptance to participate.

Sensory evaluation exercise

Ten (10) sensory judges were served with two coded samples of reconstituted Zobo concentrate (RZC) coded 615 (RZC sweetened with Sodium Cyclamate) and 617 (RZC sweetened with inverted Sugar syrup) for three weeks (21 days); and the sensory exercises were conducted on weekly basis. And the two samples of RZC were sensorily evaluated in terms of colour, aroma, taste (mouth-feel), sweetness

and overall acceptability using the nine (9) point Hedonic scale described by [25]. The sensory panelists were also served with water and cracker biscuits so that they could rinsed their mouths after tasting each of the samples for the purpose of neutralizing their taste bud. The sensory scores were then subjected to statistical analysis using T-test analysis [25,27,31,48].

Ranking test determination for sweetness

The prepared and reconstituted Zobo concentrate (RZC) were subjected to sensory evaluation using Ranking method. Ten (10) taste judges were used for the sensory evaluation of the RZC samples after being reconstituted with potable water. Every panel member continues to taste the provided samples of RZC for the 3 weeks of study based on sweetness only. Data generated were further subjected to statistical analysis as described by [25,27,31].

Microbiological status determination of the prepared ZC

Preparation of samples and culture media

Samples of ZC prepared were mixed in a Stomacher bag using a Stomacher (400 laboratory blender Type BA, 7021 Model, England, UK.). Serial dilutions were prepared, inoculated on selective media and incubated at 37°C for 24-48 hr. After incubation colonies were counted using the method of [25,58]. Also, all the culture media (i.e. Agar) used in this study was prepared according to the instructions of the respective Manufacturers.

Microbiological analysis of samples of prepared ZC

Ten (10) ml of each samples of ZC was homogenized with 90 ml sterile buffer peptone water. Further ten-fold serial dilutions of the resultant homogenates were made to obtain 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} respectively [24]. From the appropriate dilutions, 0.1 ml was plated in replicate onto different media [Saboraud Agar (SA), Potato Dextrose Agar (PDA), and Yeast Extract Agar (YEA), Oxoid Grades] using pour plate technique. At the end of the incubation periods, colonies were counted using illuminated colony counter (Gallenkamp, England). The counts for each plate were expressed as colony forming unit per ml of sample homogenate (cfu/ml) [12,18,24,25,58].

Viable bacterial cell count

After overnight incubation, growth on the Plate Count Agar (PCA) (Oxoid Grade) showing viable cells colonies of 30 to 300 were identified and counted. And the bacterial count was expressed as the number of colonies multiplied by the dilution factor [18,25,58].

Results and Discussions

Results of physicochemical analysis

This part of the study presents and discusses the results of the physicochemical values conducted on the two samples of Zobo concentrate (ZC) prepared by Hurdle Technology.

Parameters	Sample 615 (ZC Sweetened with SC)	Sample 617 (ZC Sweetened with ISS)
pH	4.1	4.3
Brix	5.8%	3.8%

Table 4: *Results of physicochemical analysis.*

Values are mean of triplicates.

Table 4 represents the results of pH and percentage Brix of the both samples of the prepared Zobo concentrate, respectively.

The pH values 4.1 and 4.3 for ZC sweetened with Sodium cyclamate and Inverted sugar syrup obtained implies that both samples of the ZC can be classified as high acid Sorrel product; and the advantage of acid food is that it does not support the survival of certain micro-organisms except some like *Lactobacillus* species [46]. The high acidity of both samples of the ZC has been reportedly attributed to the presence of naturally occurring organic acid including the Malic, Citric and Oxalic acids that are naturally present in the calyx of the *Hibiscus sabdariffa*; and this forms the basis of the malolactic fermentation process of Zobo drink [28,46].

Malic acid is known to have the ability to improve the flavour and tart-taste characteristics of food because of its sourness and acid nature and it is involved in the Krebs cycle, a process the body use to make energy; it is also used for the nutritional management of fibromyalgia, fatigue and dry-mouth because its sourness helps to stimulate and produce more saliva to help with dry-mouth, but there is no tentative scientific evidence to support the management of fibromyalgia and fatigue [1].

While, biochemical reactions in cells which involve Oxalic acid indicates that this compound is required for the formation of uracil and urotic acid owing to the fact that the former (i.e. uracil) is a component of ribonucleic acid (RNA) which is common to all cells in the human metabolism [59]. However, a study opined that Oxalic acid is a low molecular weight organic acid produced by fungi, bacteria, plants and animals and it has implications in the metabolic process of the above enumerated organisms [57]. A study has scientifically reported that Oxalic acid possess varied unrelated roles in plant metabolism, including the pH regulation in association with nitrogen metabolism, metal ion homeostasis and calcium storage during plant growth [30].

And the Citric acid, due to its acidic nature, it is predominantly used as a flavouring and preserving agent, most especially in the production of soft drinks and candies; it is also used as stabilizing agent serving as an acidulant in the production of soft drink, and also as an aid to the setting of Jams because of its general recognition as safe by the joint FAO/WHO Expert Committee on Food Additive [29]. Nonetheless, Citric acid is ubiquitous in nature because it is an intermediate in aerobic metabolism through the tricarboxylic acid (TCA) cycle where carbohydrates are oxidized to carbon dioxide.

The percentage Brix values of 5.8 and 3.8 for ZC sweetened with Sodium cyclamate and inverted sugar syrup obtained implies that both samples of the ZC are having marked different in sugar content. And this could be attributed to the fact that the ZC sweetened with Sodium cyclamate tends to show significantly high sugar content than the sample of ZC sweetened with inverted sugar syrup forming hydrolyzed Glucose and Fructose in the mixture increasing with a decrease in the optical rotation.

Result for reconstitution ratio analysis (RRA)

This part of the study presents and discusses the results of the RRA conducted on the two samples of Zobo concentrate (ZC) prepared by Hurdle Technology and respectively sweetened with Sodium cyclamate (sample 615) and inverted sugar syrup (sample 617).

Sample 615 Sweetened with SC	Sample 617 Sweetened with ISS (ml)	Potable Water (ml)	Result (ml)
10	10	30	40
100	100	300	400
1 glass	1 glass	3 glasses	4 glasses

Table 5: *Result for the reconstitution ratio analysis.*

Table 5 represent the result for RRA of the two samples of Zobo concentrate (sweetened with Sodium cyclamate (sample 615) and inverted Sugar Syrup (sample 617), respectively. The result for the RRA indicates that the ZC samples are triple strength, which is one portion of the measured volume of ZC would have to be mixed with three potions of potable of equal volume for mixing of the RZC can be for consumption.

Results for microbiological analysis

This part of the study presents and discusses the results of the microbiological status conducted on the samples of ZC prepared [21].

Samples Code	Microbial cell loads (cfu/g)	Converted Value ($log_{10}cfu^{-g}$)
615	$< 1.0 \times 10^1$	1.00
617	$< 1.0 \times 10^1$	1.00

***Table 6**: Results of the total plate count of the samples of prepared ZC.*

Table 6 presents the results of the microbiological status conducted on samples of ZC prepared and sweetened with Sodium cyclamate (Sample 615) and inverted sugar syrup (Sample 617), respectively.

Samples Code	Microbial cell loads (cfu/g)	Converted Value ($log_{10}cfu^{-g}$)
615	$< 1.0 \times 10^1$	1.00
617	$< 1.0 \times 10^1$	1.00

***Table 7**: Results of the yeasts/moulds cells count of the samples of prepared ZC.*

Table 7 presents the results of the Yeast/Moulds status conducted on samples of ZC prepared and sweetened with Sodium cyclamate (Sample 615) and inverted sugar syrup (Sample 617), respectively.

The results of the microbiological analysis of both samples of ZC show count of less than ten (10) cells counts indicating the impact of Hurdle technology adopted and used for the preparation of ZC. Hence, results of microbial analyses predict that the ZC samples are microbiologically safe for human use and consumption. Also, in addition, ZC containing Malic acid, as a highly water soluble acid, can interact with pH to inhibit the growth of yeasts, moulds and bacteria supporting the theory of the fact that anti-microbial effect of Malic acid is due to a lowering of the pH [9,60]. Also, both samples of the ZC are acidic foods that were processed by Hurdle Technology before being packaged into the primary package; and it is expected that ready-to-eat foods should not contain pathogens in them with the exception of moulds, yeasts and a few acid-tolerant bacteria that may likely grow and proliferate over period of time [58]. Hence, this study established that microbial cells that were detected from the two samples of the ZC (Table 6 and 7) are within the satisfactory (10^3 cfu/g) and marginal (10^3 to $< 10^5$ cfu/g) microbial limits for RTE foods [20,24].

Results of sensory evaluation

This part of the study presents and discusses the results of the sensory evaluation conducted on the samples of ZC reconstituted.

Sensory attributes	Cal. Values	Tab. Value	Conclusion
Colour	-5.89	2.262	NSD
Aroma	3.007	2.262	SD
Taste (mouth feel)	-2.276	2.262	NSD
Sweetness	4.237	2.262	SD
Overall acceptability	-2.006	2.262	NSD

***Table 8**: Result of t-test of the sensory evaluation conducted on both samples of RZC.*

Keywords: NSD = No Significant Difference; SD = Significant Difference.

Table 8 presents the sensory attributes of samples of RZC sweetened with Sodium cyclamate (Sample 615) and inverted sugar syrup (Sample 617), respectively.

The result of the sensory evaluation Table 8 suggests that there is a significant difference in some sensory parameters including aroma and sweetness; but there is no significant difference in the other sensory attributes including colour, taste (i.e. mouth-feel) and overall acceptability, respectively ($P < 0.05$). This is owing to the fact that when T-calculate is greater than the T-tabulated it indicates that there is a significance difference between the two samples; while if the T-calculate is less than the T-tabulated it implies that there is no significant difference between the two samples under investigation [25,27,31]. Thus in this study, both samples of RZC have similar sensory attributes of colour, taste (i.e. moth-feel) and overall acceptability ($P < 0.05$), and as well both samples of RZC have no similar organoleptic attributes of aroma and sweetness ($P < 0.05$).

Results for sensory evaluation (Ranking test)

Sample code	Ranking	SD	Remark
615	25	15 - 25	SD
617	18	16 - 24	SD

Table 9: *Result for sensory evaluation (Ranking test).*

Keywords: SD = Significant Difference; NSD = No Significant Difference; SD = Standard Deviation.

Table 9 represents the results for ranking test analysis of the two samples of Zobo concentrate sweetened with Sodium cyclamate and inverted sugar syrup, respectively.

Applying the rapid ranking analysis to establish which of the RZC is sweeter than the other; the rank totals are compared with the values shown in table 9. There are ten replications and two samples of RZC were analysed using human organs for sweetness. Hence the Tabular entries are 15 - 25 (upper entry) and 16 - 24 (lower entry). The lower rank sum of the upper entry which are to be used since we do not have a reference product is 15. And the highest rank sum is 25. However, since 18 (the rank sum for sample 617) is higher than 15 (i.e. the upper left value in the block from table 9) and 25 (the rank sum of sample 615) is equal to 25 (i.e. the upper right value in the block from table 9), this indicates that there is significance difference ($p > 0.05$) level significance in sweetness of the both samples of RZC sweetened with the Sodium cyclamate and inverted sugar syrup, respectively. From the table 9 above, the ranking results indicates that there is significance ($P < 0.05$) difference in the sweetness of the two samples of RZC because of the different types of sweeteners used for sweetening both samples of ZC, respectively.

Conclusion

In summary, this research demonstrates that deep dark purple Sorrel calyx was used to prepare two samples of triple strength Zobo concentrate (ZC) using Hurdle Technology and sweetened with Sodium cyclamate (sample 615) and inverted sugar syrup (sample 617); the results of sensory, microbial and physico-chemical analyses indicated that ZC can be prepared successfully for human use and consumption. Thus, both samples of the ZC have similar microbiological, sensory and physico-chemical properties. And the prepared ZC would be used for the production of the Instant Zobo drink by Spray drying method.

Acknowledgement/Special Notification

We sincerely appreciate TetFund, Nigeria for sponsoring this study. Also, this developed product (i.e. Triple strength Zobo Concentrate ready for spray drying) is on the process of patenting; hence no person or group of persons is allowed to use the Process Flow Diagram and/or the Recipes formulation reported in this study.

Authors Contribution to the Study

All the authors designed and performed the study; also the three authors prepared and approved the draft and final manuscripts. And there was no conflict of interest regarding this research throughout the study.

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